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An improved validated RP-HPLC method for simultaneous estimation of Metformin and Dapagliflozin from finish dosage form.

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Abstract

A simple and precise reversed-phase high-performance liquid chromatography method was developed and validated for the simultaneous determination of **Metformin** (MET) **hydrochloride** and **Dapagliflozin** (DAPA) in bulk and pharmaceutical dosage form.

Chromatography was carried out on **Zorobax Eclipse Plus Phenyl Hexyl C18 (250 mm × 4.6 mm, 5 µ particle size)** column containing mobile phase of **Buffer:Methanol:Acetonitrile** in the ratio of **40:35:25% v/v.** (1ml of **Triethylamine** is added and **pH** is **adjusted to 3.7** with **0.1% Orthophosphoric acid**) at a flow rate of **1.0 ml/minute**. The analyte was monitored using **Photo Diode Array Detector** (PDA) at **267 nm**.

The retention time was found to be **2.63 minutes and 9.34** minutes for **MET hydrochloride** and **DAPA** respectively. The proposed method was found to be having **linearity** in the concentration range of 250-750 µg/ml for **MET** (**R2= 0.99938**) and 5-15 µg/ml for **DAP** (**R2= 0.99901**), respectively. The mean % **recoveries** obtained were found to be **98.00-102.00%** for **MET** and **98.00 -102.00%** for **DAPA** respectively. The method developed has been statistically validated according to ICH guidelines.

The results of analysis have been validated as per **International Conference on Harmonization** (ICH) guidelines. Hence the optimized method can be successfully applied for the simultaneous determination of Metformin hydrochloride and Dapagliflozin in the routine quality control analysis. Significance of developed method is that, it can be utilized for routine or unknown sample analysis of assay of Metformin HCl and Dapagliflozin in pharmaceutical dosage form developed by various Pharmaceutical Industry. The Proposed method was found to be rapid, accurate, precise, specific, robust, rugged and economical.

Keywords: Dapagliflozin, Metformin Hydrochloride, RP-HPLC,

INTRODUCTION

Metformin (MET) hydrochloride (Fig. 1) is chemically known as (3-(diaminomethylidene)-1,1-dimethylbiguanide; hydrochloride. It has molecular formula $C_4H_{11}N_5$ and molecular weight is 129.16 g/mol. MET is an agent belonging to the biguanide class of antidiabetics with

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antihyperglycemic activity. MET is the first line agent for the treatment of Type 2 diabetes.

Dapagliflozin (DAPA) (Fig. 2) is a selective sodium glucose cotrasporter subtype-2 (SGLT-2) inhibitor with antihyperglycemic activity. Its chemical name is (2S, 3R, 4R, 5S, 6R)-2[4-chloro-3-(4-ethoxyphenyl) methyl] phenyl-6-(hydroxymethyl)oxane-3,4,5-triol. It has molecular formula of $C_{21}H_{25}ClO_6$ and molecular weight is 408.9 g/mol. DAPA is a SGLT-2 inhibitor indicated for managing diabetes mellitus Type 2.

The combination dosage form selected for the present study contains DAPA and MET in solid oral dosage forms. Recently this combination has been approved by USFDA. The main aim of this study was to develop a stability indicating method for the simultaneous estimation of DAPA and MET in bulk and to apply the developed method for the quantitative determination of these drugs from its tablets and the reverse phase high performance liquid chromatography (RP-HPLC) method is chosen. This method is validated as per International Conference on Harmonization (ICH) guidelines. Literature survey revealed that some analytical methods were reported for the estimation of DAPA and MET individually or in combination with other drugs, by HPLC analytical method. No of stability indicating RP-HPLC method was reported for estimation of both these drug. Now a day, stability indicating method as important regulatory and CGMP point of view to assess the drug stability. In the present study, it was tired to develop stability indicating RP-HPLC method to determine possible degradation products of DAPA and MET.

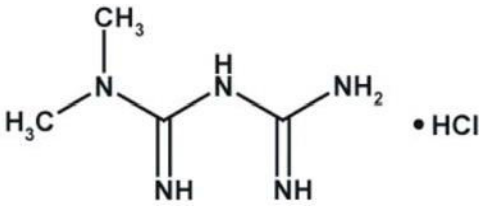


Fig. 1: Structure of Metformin

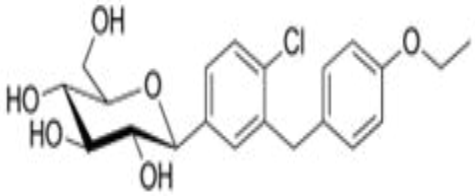


Fig. 2: Structure of Dapagliflozin

MATERIALS AND METHODS

Instrument

Chromatographic separation is achieved using HPLC System (Agilent HPLC 1100Series) containing Quaternary pump solvent manager. An autosampler and PDA detector. The output Signal is monitored and processed using Ezchrom Elite Software. An Zorobax eclipse Plus Phenyl Hexyl (250 x 4.6mm, Id 5µ) is used as the stationary phase. Isocratic Mobile phase is used.

Chemicals and reagents

Methanol,Acetonitrile, Triethylamine & 0.1% Orthophosphoric acid, Water, and potassium dihydrogen ortho-phosphate buffer of HPLC grade were obtained from Merck Ltd.

Preparation of Buffer pH 3.7

Dissolve 3.4 g of potassium dihydrogen ortho-phosphate in 1000 mL of HPLC grade water. 1ml **Triethylamine** is added and Sonicated to dissolve and pH is adjusted to 3.7 with 0.1% ortho phosphoric acid and filtered through 0.45µ filter.

Mobile phase (Diluent) Preparation:
400mL of Buffer pH 3.7, 350mL Methanol & 250 mL Acetonitrile Mixed well and degassed.

Chromatographic Condition.

HPLC System	:	Agilent 1100 Series with PDA Detector
Mobile Phase	:	**Buffer :Methanol:Acetonitrile (40:35:25% v/v)**
Column Used	:	Zorobax eclipse Plus Phenyl Hexyl (250X4.6mm, id 5µ)
Column Temperature	:	30°C
Flow Rate	:	1.0mL/Minutes
Injection Volume	:	20µL
Detection Wavelength	:	267nm
Run Time	:	15minutes

Preparation of Stock (Standard) Solution
Weigh and transfer about 100 mg of standard Metformin in 100 ml volumetric flask. Add about 30 ml of diluent and sonicate to dissolve. Cool the solution and dilute up to the mark with diluent (1mg/mL=1000ppm).

Weigh and transfer about 100 mg of standard **Dapagliflozin** in 100 ml volumetric flask. Add about 30 ml of diluent and sonicate to dissolve. Cool the solution and dilute up to the mark with diluent (1mg/mL=1000ppm).
Pipette out standard 10ml of **Dapagliflozin** solution and transfer in 50ml volumetric flask and dilute up to the mark with diluent (0.2mg/mL= 200ppm).

Sample solution Preparation (Control Sample)
Weigh & transfer 20 tablets in mortal pester previously cleaned. Crush the tablets to fine powder. Weigh and transfer the powder equivalent to 100mg of **Metformin** to 100 ml volumetric flask. Add 70 ml of diluent and sonicate for 15mins with intermittent shaking. Cool the solution and dilute up to the mark with diluent. Filter through 0.45micron nylon syringe filter, first few ml of **Metformin** is discarded and collected as **stock sample solution**.

Weigh & transfer 20 tablets in mortal pester previously cleaned. Crush the tablets to fine powder. Weigh and transfer the powder equivalent to 100mg of **Dapagliflozin** to 100 ml volumetric flask. Add 70 ml of diluent and sonicate for 15mins with intermittent shaking. Cool the solution and dilute up to the mark with diluent. Filter through 0.45micron nylon syringe filter, first few ml of

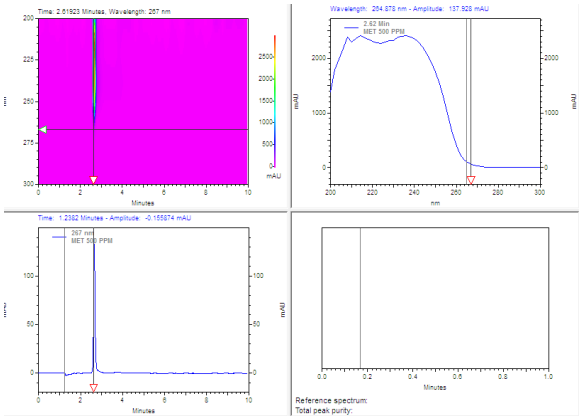
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, 2023

Dapagliflozin is discarded and collected as **stock sample solution.**

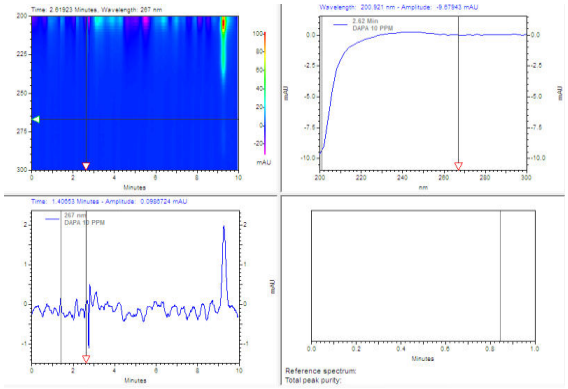
Pipette out standard 10ml of Dapagliflozin solution and transfer in 50ml volumetric flask and dilute up to the mark with diluent (0.2mg/mL= 200ppm).

Selection of wavelength

Scan the standard solution in HPLC between 200 nm and 400 nm on spectrum scan mode, The two drugs show λmax at 267 nm for DAPA and MET.



Wavelength scan for Metformin



Wavelength scan for **Dapagliflozin**

Method validation

Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of drug in samples within a given range.

From standard stock solution of MET & DAPA shown in table no- 01, the different concentration of solution is prepared by using **calibrated micropipette (10- 100** µL & **100- 1000** µL pipette). The final concentration of this solution was observed in the range of 5–15 µg/mL for DAPA and 250–750 µg/mL for MET. Calibration curves were plotted with observed peak areas against concentration to obtain the calibration curve and correlation coefficients. Characteristics parameters for regression equation (y = mx+c) of the method and these parameter were used to confirm the good linearity of the method. The results are shown in Table no 2 and 3 and Graph 1 and 2.

Sr No	Solution %	Met Conc In Ppm	Met Conc In Ppm	Met Std µl	Dapa Std µl	Diluent µl	µl
1	50%	250	**5**	**250**	**50**	**700**	1000
2	80%	400	**8**	**400**	**80**	**520**	1000
3	90%	450	**9**	**450**	**90**	**460**	1000
4	100%	500	**10**	**500**	**100**	**400**	1000
5	110%	550	**11**	**550**	**110**	**340**	1000

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, 2023

6	120%	600	**12**	**600**	**120**	**280**	1000
7	150%	750	**15**	**750**	**150**	**100**	1000

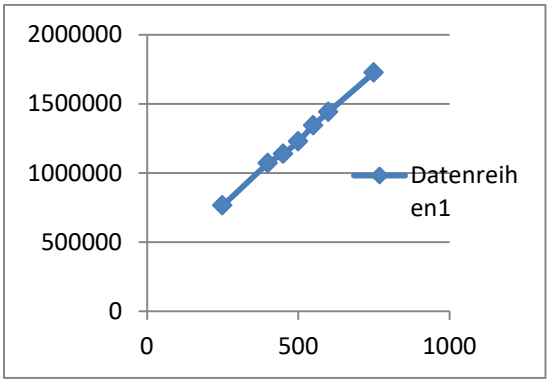
Table no 1

SR NO	SOLUTION %	Met CONC IN ppm	AREA
1	50%	250	**763923**
2	80%	400	**1069020**
3	90%	450	**1137814**
4	100%	500	**1228259**
5	110%	550	**1343818**
6	120%	600	**1441204**
7	150%	750	**1725207**
		CORRELATION	**0.99938**

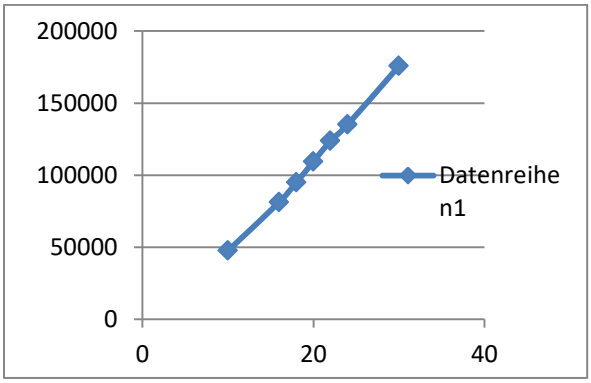
Table no 2

SR NO	SOLUTION %	Dapa Conc in ppm	AREA
1	50%	5	**47542**
2	80%	8	**81286**
3	90%	9	**94840**
4	100%	10	**109429**
5	110%	11	**123759**
6	120%	12	**135106**
7	150%	15	**175757**
		CORRELATION	**0.99901**

Table no 3



Graph 1: Calibration curve of Metformin



Graph 2: Calibration curved of Dapagliflozin

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as present recovery by the assay of known added amounts of analyte.

From standard stock solution of MET & DAPA shown in table no- 04, the different concentration of solution is prepared by using **calibrated micropipette (10- 100** µL & **100- 1000** µL pipette).

The accuracy was determined by DAPA and MET (equivalent to 500 mg of MET and 10 mg of DAPA) (50%, 100%, and 150% of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. The resulting mixtures were analyzed in triplicates. The % recovery of added drug is taken as a measure of accuracy. The results are shown in Table 5 & 6.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, 2023

Sr No	Solution %	Met Conc In Ppm	Met Conc In Ppm	Met Std µl	Dapa Std µl	Diluent µl	µl
1	**100% STD**	**500**	10	**50**	**500**	450	1000
2	50%	250	5	**25**	**250**	725	1000
3	100%	500	10	**50**	**500**	450	1000
4	150%	750	15	**75**	**750**	175	1000

Table no 4

	MET	wt in mg	Conc.	ppm
	Std wt.	100.0	**Std Conc**.	500
	Sample wt.	100.0	50 % spl conc.	250
			100 % spl conc.	500
			150 % spl conc.	750
No of Inj.	**std area**	**50% spl area**	**100% spl area**	**150% spl area**
Inj-1	1323215	672196	1302743	1995752
Inj-2	1332986	670163	1306981	1984694
Inj-3	1343543	674086	1313176	1971308
Inj-4	1338391			
Inj-5	1318029			
Mean	**1331233**	**672148**	**1307633**	**1983918**
STDEV	10541.3	1961.9	5247.0	12240.5
RSD	0.792	0.292	0.401	0.617
%		100.98	98.23	99.35
	Formula =	$\frac{\text{Sample area x std conc.}}{\text{Std area x sample conc.}}$	X 100	

Table no 5

	DAPA	wt in mg	Conc.	ppm
	Std wt.	100.0	std conc.	10
	Sample wt.	100.0	50 % spl conc.	5
			100 % spl conc.	10
			150 % spl conc.	15
No of Inj.	**std area**	**50% spl area**	**100% spl area**	**150% spl area**
Inj-1	60720	30250	59880	92300
Inj-2	61013	31158	60568	93002
Inj-3	61598	30550	60694	91564
Inj-4	60008			
Inj-5	61490			
Mean	**60966**	**30653**	**60381**	**92289**

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, 2023

STDEV	643.1	462.6	438.1	719.1
RSD	1.055	1.509	0.726	0.779
%		100.56	99.04	100.92
	Formula =	$\frac{\text{Sample area x std conc.}}{\text{Std area x sample conc.}}$ X 100		

Table no 6

Results and Discussion

Linearity

Table. 2&3 Linearity Results

The retention time was found to be **2.63 minutes and 9.34** minutes for **MET hydrochloride** and **DAPA** respectively. The proposed method was found to be having **linearity** in the concentration range of 250-750 µg/ml for **MET** (**R2= 0.99938**) and 5-15 µg/ml for **DAP** (**R2= 0.99901**), respectively.

Table.4 Acceptance criteria.

Parameters	**Observed Results**	**Acceptance Criteria**
Correlation Coefficient of MET	0.99938	NLT 0.99
Correlation Coefficient of DAPA	0.99901	NLT 0.99

Results:

It was observed that the proposed method was found to be having **linearity** in the concentration range of 250-750 µg/ml for **MET** and 5-15 µg/ml for **DAP** respectively.

Accuracy

Table.5&6 Accuracy Results

The mean % **recoveries** obtained were found to be **98.00-102.00%** for **MET** and **98.00 -102.00%** for **DAPA** respectively.

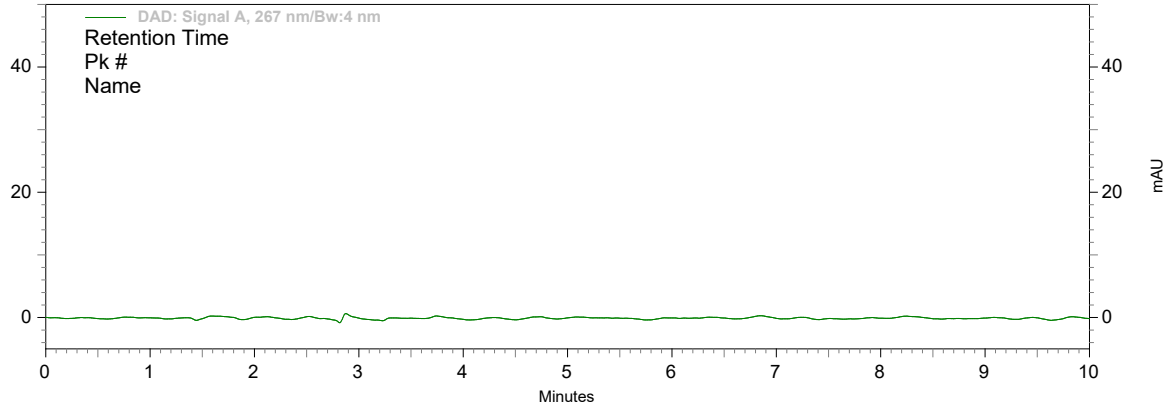
Table.4 Acceptance criteria.

Parameters	Observed Results	Acceptance Criteria
Accuracy of MET	50%-100.98% 100%-98.23% 150%-99.35%	Between 98% to 102%
Accuracy of DAPA	50%-100.56% 100%-99.04% 150%-100.92%	Between 98% to 102%

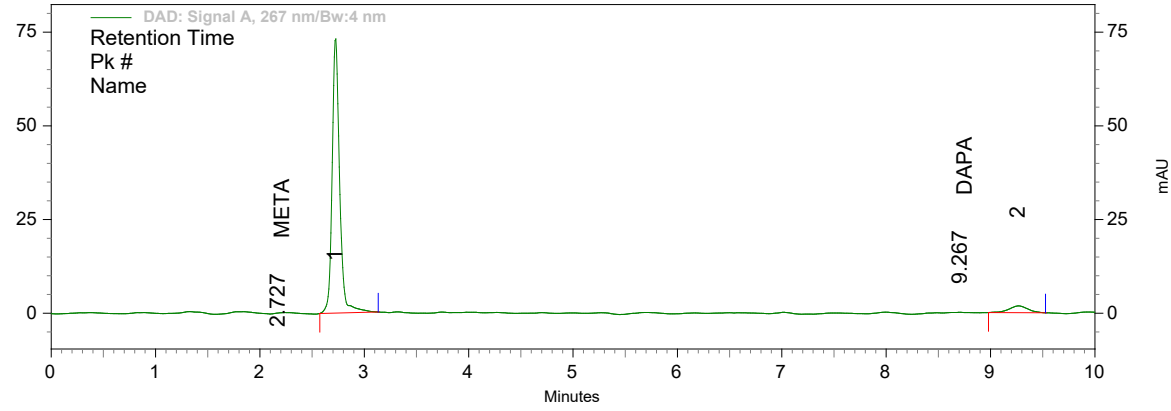
Results:

It was observed that the mean % **recoveries** obtained were found to be **98.00-102.00%** for **MET** and **98.00 -102.00%** for **DAPA** respectively.

The representative chromatogram obtained for blank in linearity shown in figure 1.

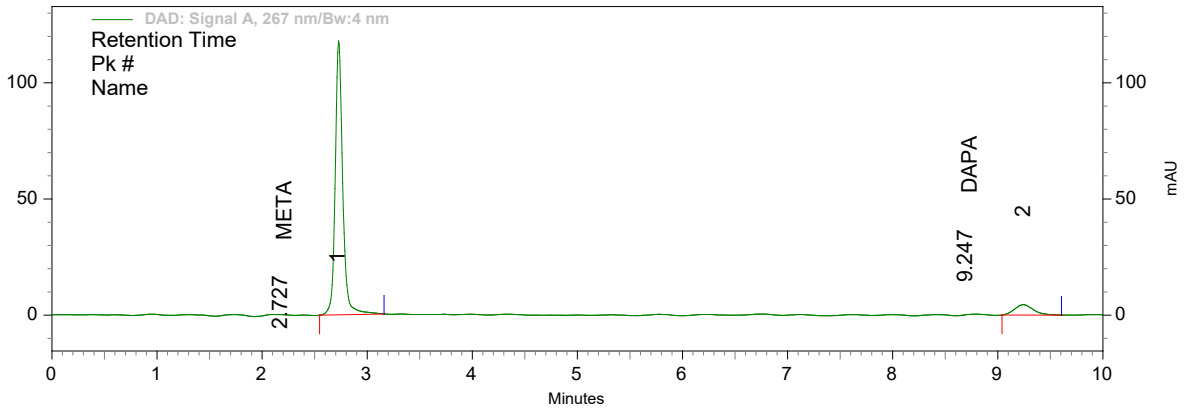


The representative chromatogram obtained for standard solution shown in linearity of MET & DAPA in figure 2.

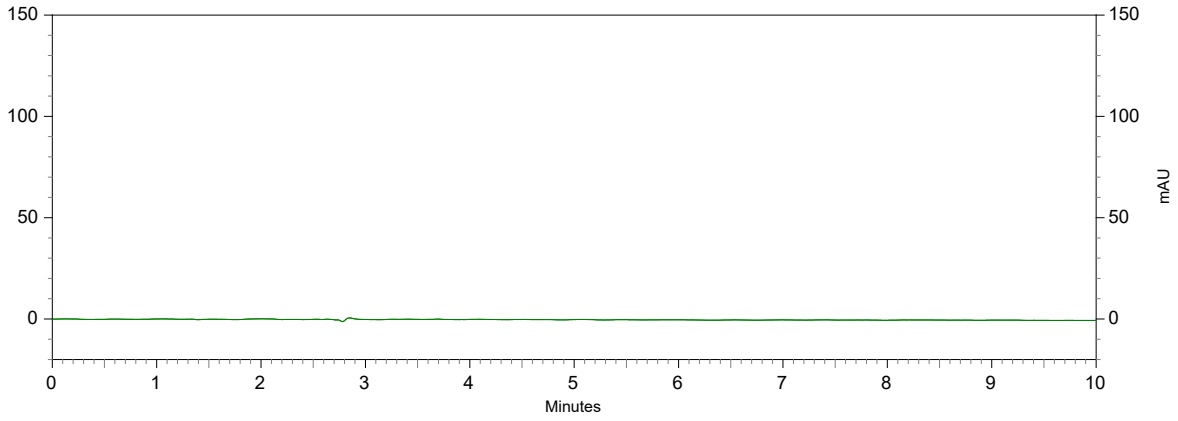


ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, 2023

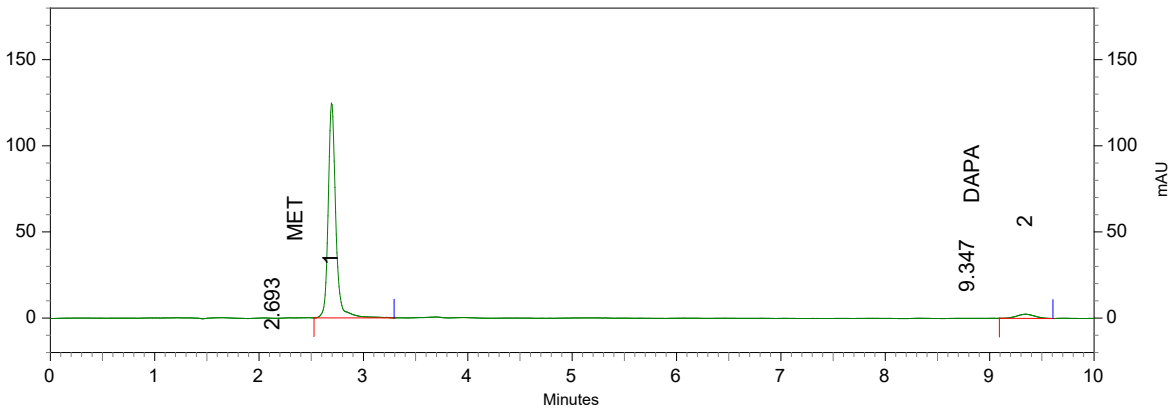
The representative chromatogram obtained for Control sample solution shown in linearity of MET & DAPA in figure 3.



The representative chromatogram obtained for blank in accuracy shown in figure 4.

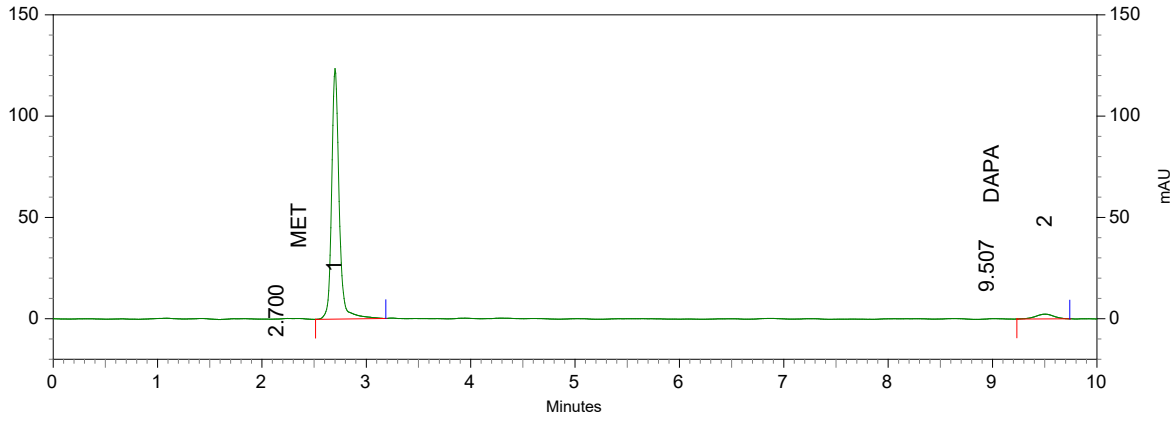


The representative chromatogram obtained for standard solution shown in accuracy of MET & DAPA in figure 5.



ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, 2023

The representative chromatogram obtained for Control sample solution shown in accuracy of MET & DAPA in figure 6.



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